Energy and Environment Research; Vol. 2, No. 1; 2012
ISSN 1927-0569 E-ISSN 1927-0577
Published by Canadian Center of Science and Education

Assessment of Metal Concentrations, Chlorophyll Content and Photosynthesis in *Phragmites australis* along the Lower Diep River, CapeTown, South Africa

Olutoyosi Ayeni[1], Patrick Ndakidemi[2], Reinette Snyman[3] & James Odendaal[1]

[1] Department of Environmental and Occupational Studies, Cape Peninsula University of Technology, South Africa

[2] School of Life Sciences and Bio-engineering, The Nelson Mandela African Institute of Science and Technology, Arusha, Tanzania

[3] Department of Biodiversity and Conservation, Cape Peninsula University of Technology, South Africa

Correspondence: Olutoyosi Ayeni, Department of Environmental and Occupational studies, Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 652, Cape Town 8000, South Africa. Tel: 27-732-657-895. E-mail: ayeniolaide@yahoo.com

Received: November 14, 2011 Accepted: November 30, 2011 Online Published: May 17, 2012

doi:10.5539/eer.v2n1p128 URL: http://dx.doi.org/10.5539/eer.v2n1p128

Abstract

Phragmites australis growing at 4 selected sites along the bank of the lower Diep River, Cape Town, South Africa and in adjacent soil was assessed for photosynthesis, chlorophyll content and metal concentrations in shoots and roots. The rate of photosynthesis was determined using the Infra - red gas analyzer (IRGA). Chlorophyll content of leaves was determined by extraction of the pigments using dimethyl sulphoxide (DMSO). Chlorophyll was quantified from the extract using a spectrophotometer (645 and 665 nm). Ten metals (cadmium, copper, iron, lead, manganese, nickel, zinc, aluminium, chromium, and cobalt) in the shoots and roots of *Phragmites australis* were measured by ICP-MS. The abundance of the metals were in the order Al > Pb > Cd > Co > Ni > Cr; and for micronutrients, Fe > Mn > Zn > Cu both in the shoots and roots from all the sites investigated. Chlorophyll a, b and total chlorophyll ($Chl._T$), as well as photosynthesis were significantly lower in *P. australis* from the river bank compared with the adjacent soil. Increased metal loads in plants from the river bank were found to accompany the decreasing chlorophyll concentrations and photosynthetic rate. More metals were found to be accumulated in plants on the river bank compared to plants from adjacent to the river bank.

Keywords: metals, *phragmites*, chlorophyll content, photosynthesis

1. Introduction

A cornerstone of ecotoxicological science is the ability to demonstrate a relationship between the exposure of metals and the physiological responses in plants (such as changes in chlorophyll content and photosynthesis activities). The demonstration of a metal exposure-response relationship is an essential criterion for establishing that the metal is responsible for the effects measured. For most metals, exposure to low levels does not lead to any observable effect, but it is only after a threshold is reached that an effect can be detected (Kabata-Pendias & Pendias, 1992; Rodríguez et al., 2007).

Metal toxicity is one of the common stresses that limit plant growth and development (Gallego et al., 1996; Liphadzi & Kirkham, 2006). Scientific evidence has shown that plant species which grow in polluted environments may be stressed in various ways. For example, bioaccumulation of metals to toxic concentrations (through direct uptake by the plants' roots, stems or shoots) results in malfunctioning of their physiological systems (Dahmani-Muller et al., 2000; Monni et al., 2001; Plekhanov & Chemeris, 2003; Liphadzi & Kirkham, 2006).

If a plant is stressed, changes in the chlorophyll content may occur before any physical signs of stress are evident. Several cases of decreased chlorophyll synthesis and metabolism due to metal toxicity have been reported in the plant kingdom (Abdurakmanova et al., 2000; Schoefs, 2001; Kucuk et al., 2003; Calheiros et al., 2007; Baldantoni et al., 2009; Bragato et al., 2009; Bonanno & Lo Giudice, 2010).

Zayed et al. (1998) observed chlorosis and reduced growth in duckweed at higher levels of metals such as Fe, Cu, Cd, Hg, Pb, Ni, Zn and Mn. Similarly, studies by Stobart et al. (1985) and Dong et al. (2005) on the effects of cadmium on chlorophyll content in barley, revealed inhibited biosynthesis, reduction in total chlorophyll content and the chlorophyll a/b ratio.

Chlorophyll concentration may fundamentally influence the functioning of the photosynthetic apparatus and thus affect whole plant metabolism (Clijsters & van Assche, 1985; Sun & Wu, 1998; Prasad & Strzalka, 2000). For example, rye grass *(Lotium perenne,* cv.S-23) leaves turned yellowish when treated with Ni, plants became necrotic and suffered interveinal chlorosis (Khalid & Tinsley, 1980). Sheoran et al. (1989) reported a reduction in photosynthesis and enzyme activities in pigeon pea (*Cajanus cajan*) indirectly by reporting a decrease in chlorophyll content due to elevated levels of cadmium and nickel in the leaves. In higher plants, studies have shown that growth and photosynthetic activities were significantly affected by cadmium (Nagel et al., 1996).

Most of the published data concerning toxicity testing of metals has focused on single metal effects. However, metal pollution of plants growing in polluted environments in nature has been shown to be due to the presence of cocktail of several metals (Walker et al., 2003; Rodríguez et al., 2007). They may exhibit toxicity simultaneously and interactively at different levels (Vάzquez et al., 2006). It is therefore worthwhile to assess metal toxicity induced to plants through expression of biomarkers such as those related to chlorophyll synthesis and photosynthetic activity.

The Diep River originates from the Kasteel Mountain, Malmesbury and flows in a south-westerly direction towards Table Bay, where it flows into the Atlantic Ocean (Brown & Magoba, 2009). Previous studies have shown that the Diep River is polluted in terms of metals (Ayeni et al., 2010; Shuping et al., 2011). In the present study, the extent to which physiological parameters in *Phragmites australis* (chlorophyll content, photosynthesis) and metal accumulation are affected at different sites along a pollution gradient in Diep River in Cape Town.

P. australis is an emergent macrophyte, cosmopolitan in distribution and the most productive natural plant population in the biosphere (Wetzel, 1995; Cronk and Fennessy, 2001; Saltonstall, 2008). It grows perennially, with a constant turnover of population members that are senescing as new cohorts. Reeds (*P. australis*) are the focus of this study because it is the most abundant plant species lining the river banks along the Diep River. Furthermore, it is widespread along South African rivers and commonly occurs in monospecific stands (Laing et al., 2003; Saltonstall, 2008).

2. Materials and Methods

2.1 Site Selection

The study was conducted along the banks of the lower Diep River. This river is located in Cape Town, Western Cape, South Africa. The four sampling sites were selected based on their location near both an oil refinery and landfill site, which may emit a cocktail of pollutants to some distance in the environment.

2.2 Collection and Preparation of Plant Samples

For each of the sites, plant specimens were collected from the river bank and adjacent soil (2 m apart) in the same vicinity. Whole plants were completely uprooted and were then separated into shoots (stems and leaves) and roots. In the laboratory, the roots and shoots were then washed thoroughly with distilled water to remove any soil particulate matter and then dried in the oven at 60ºC for 48 h, after which they were ground with a mortar and pestle. Each of the samples was then sieved using a nylon sieve (2 mm pore size).

2.3 Digestion of Plant Samples

This was carried out as described by Odendaal and Reinecke (1999). For the digestion process 10 ml of 55% HNO_3 was added to each sample (1 g) in test tubes and stirred properly using a glass rod. Each of the mixtures was then heated on a Universal Block Dryer (UBD) heater in a fume cupboard at 40ºC for 1 h, then at 120 ºC for 3 h. The samples were then allowed to cool to room temperature. Each of the cooled solutions was made up to 20 ml with distilled water and was filtered using cellulose nitrate filter paper (0.45 µm). Each of the filtrates obtained were further diluted to a final volume of 100 ml with distilled water, appropriately labelled and stored in the refrigerator.

2.4 Metal Analysis

The digested plant samples were analyzed for the presence of metals (Al, Cr, Co, Ni, Cd, Pb, Mn, Fe, Cu and Zn) using the Inductively Coupled Plasma - Mass Spectrophotometer (7500 CE, Agilent, England). To obtain the plant metal concentrations, the ICP values were converted using the formula:

$$PMC = \frac{(ICP\ reading - blank\ reading)\ \mathrm{X}\ dilution\ factor(20)}{WPS(mg)}$$

Where: PMC = Plant metal concentration (mg kg^{-1}); ICP = Inductively Coupled Plasma values; WPS = Weight of plant sample (g). The plant metal concentration data were statistically analyzed using the STATISTICA software package 2009 (StatSoft Inc., Tulsa, OK, USA).

2.5 Determination of Photosynthesis in Plant Leaves

Measurements were taken on-site using an Infra-red gas analyzer (IRGA, Pharmacia, LKB. Ultraspec.11E, Blockrom, England). Photosynthetic parameters in the intact plant leaves were assessed in the morning between 8am and 11am, according to the manufacturer's instructions. Parameters measured included photosynthesis (A), rate of evapo-transpiration (E), intercellular carbon dioxide concentration (Ci) and stomata conductance (Gs). Readings were recorded at 20 minute intervals. The carbon dioxide assimilation rate was expressed as the amount of carbon dioxide assimilated per unit leaf area and time (μmol) carbon dioxide was consumed.

2.6 Determination of Chlorophyll Contents in Plant Leaves

Plant leaves were neatly detached using a gloved hand and placed into a polythene bag, sealed, appropriately labeled and placed in a cooler. Samples were then transported to the laboratory. The leaves were cut into pieces using a clean scalpel, macerated in a crucible and then used for chlorophyll contents analysis as described by Hiscox and Israelstam (1979). To 100 mg of macerated leaves, 7 ml dimethyl sulphoxide (DMSO) was added in a vial and incubated at 4°C for 72 h. After incubation, the extracts obtained were diluted with 10 ml DMSO; 3 ml of the diluted extract was withdrawn, transferred into cuvets and the absorbance determined at 645 and 663 nm. A pure solution of dimethyl sulphoxide (DMSO) was used as blank. Total chlorophyll contents (Chlorophyll a and b) were determined using the equation below as described by Arnon (1949) and results were expressed as mg L^{-1}.

Chlorophyll a: Chl a = $12.7D_{663}$ - 2.69 D_{645}

Chlorophyll b: Chl b = $22.9D_{645}$ - $4.68D_{663}$

Chlorophyll Total: (ChI.τ) = 20.2 D_{645} + 8.02 D_{663}

3. Results

3.1 Metals in Plant Shoots and Roots

Results of metal concentrations in the shoots and roots of *P. australis* collected from both the river bank and the adjacent soil from site 1 to site 4 are shown in Table 1.

Table 1. Concentrations ($mg.kg^{-1}$) of metals in plant shoots from four different soil sites in the Diep River

Site	Status of the sampled site	Al	Cr	Co	Ni	Cd	Pb	Mn	Fe	Cu	Zn
						mg.kg^{-1}					
1	Adjacent soil	8.8±1.1^{b}	0.0±0.0^{b}	0.1±0.0^{a}	0.01±0.0^{b}	2.3±1.9^{b}	0.6±0.0^{b}	0.8±0.7^{b}	20.4±11.7^{a}	6.0±1.9^{a}	0.0±0.0^{b}
	River bank	23.7±8.7^{a}	0.1±0.01^{a}	0.1±0.0^{a}	0.3±0.2^{a}	9.0±8.9^{a}	2.4±0.8^{a}	1.6±1.1^{a}	26.3±20.7^{a}	6.0±1.9^{a}	3.8±2.8^{a}
	F- statistics	2.91*	0.24***	0.36^{NS}	26.43*	10.64**	5.85***	566.55**	1.05^{NS}	9.09***	121.26***
2	Adjacent soil	8.7±1.0^{a}	0.01±0.0^{b}	0.0±0.0^{b}	0.03±0.0^{b}	0.01±0.0^{b}	0.5±0.0^{b}	2.9±0.6^{b}	23.6±2.2^{b}	0.0±0.0^{b}	5.1±1.4^{b}
	River bank	23.6±4.5^{b}	0.2±0.11^{a}	0.1±0.0^{a}	0.5±0.1^{a}	56.6±53.7^{a}	1.5±0.6^{a}	12.1±3.4^{a}	43.4±17.7^{a}	2.6±0.4^{a}	11.7±3.8^{a}
	F- statistics	10.43***	2.45*	63.28***	39.38***	0.73*	2.68*	73.57***	1.23*	44.25***	2.67*
3	Adjacent soil	16.9±6.2^{b}	0.8±0.8^{b}	0.0±0.0^{b}	0.3±0.3^{b}	0.01±0.0^{b}	1.4±0.3^{b}	18.5±6.6^{b}	26.2±3.0^{b}	2.4±1.2^{b}	5.3±0.6^{b}
	River bank	56.6±53.7^{a}	1.18±1.07^{a}	0.35±0.18^{a}	1.9±0.4^{a}	0.2±0.0^{a}	23.6±2.2^{a}	33.4±5.0^{a}	33.8±5.4^{a}	3.19±0.8^{a}	18.7±4.6^{a}
	F- statistics	0.40*	537.63*	29.7*	10.60***	1.71*	0.60*	3.21*	1.49*	439.00***	8.70***
4	Adjacent soil	10.1±8.9^{b}	0.0±0.0^{a}	16.9±6.2^{b}	0.6±0.6^{b}	0.0±0.0^{a}	1.3±1.3^{b}	35.9±5.4^{b}	6.3±6.3^{b}	0.3±0.3^{b}	10.1±2.60^{b}
	River bank	237.9±21.8^{a}	0.0±0.0^{a}	55.9±53.6^{a}	0.8±0.8^{a}	0.0±0.0^{a}	16.9±3.0^{a}	97.3±5.7^{a}	56.6±53.7^{a}	3.9±1.1^{a}	50.6±6.6^{a}
	F- statistics	0.56*	2.25^{NS}	0.40*	10.8**	2.25^{NS}	23.47***	60.59***	0.59*	773.46*	25.97***

Mean ± SE, followed by dissimilar letter in the same column are significant at P=0.05 according to Fischer LSD. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant)

3.1.1 Aluminium (Al)

Al concentration at all sites was significantly higher in shoots collected from the river bank compared with those from adjacent soil. The mean values for samples collected from adjacent soil ranged from 8.7-16.9 mg.kg^{-1}, whereas those from the river bank ranged from 23.6 - 237.9 mg.kg^{-1}. The highest Al concentration in plant tissues were found in samples collected from site 4 and 3 respectively (Table 1). The Al concentration in the roots ranged from 1.1-313 mg.kg^{-1} (Table 1). Three out of four sites had Al levels in the roots which were significantly higher from samples collected from the river bank relative to those from adjacent soil. A higher level of Al was detected in roots taken from sites 4, 2, 3 and 1 respectively.

3.1.2 Chromium (Cr)

Significant differences were observed in Cr concentrations between plant samples of *P. australis* collected along the river bank and from the adjacent soil at sites 1, 2 and 3 (Table 1). Cr was not detected in shoots sampled from site 4. The concentrations of Cr in shoots collected from the adjacent soil were in the range of 0.0-0.8 mg.kg^{-1}. From the river bank, the shoot concentration of Cr varied from 0.1-0.8 mg.kg^{-1}. In the roots, Cr was detected in plants from two sites out of the four. The values from the river bank ranged from 0.1-488.6 mg.kg^{-1}, while from adjacent soil the concentration ranged from 0.0-12.5 mg.kg^{-1} (Table 1). Plants from site three had roots with higher concentrations of Cr as compared with site two.

3.1.3 Cobalt (Co)

Shoot concentration of Co in *P. australis* plants from the river bank ranged from 0.1-55.9 mg.kg^{-1} (Table 1), while those from the adjacent soil had values ranging from 0.0-16.9 mg.kg^{-1}. Three out of the four shoot samples collected from the river bank had Co which was significantly higher than those collected from adjacent site (Table 1). Roots from the river banks had Co concentrations ranging from 0.2-25.6 mg.kg^{-1}, and the lowest values ranging from 0.0-0.2 mg.kg^{-1} were reported from the adjacent soil investigated. All roots from the river bank from the 4 sites contained Co which was significantly higher than those in adjacent soil (Table 1).

3.1.4 Nickel (Ni)

P. australis shoot samples collected from the river bank had consistently higher Ni concentrations in all four sites with values ranging from 0.3-1.9 mg.kg^{-1}. Ni was lowest in shoots sampled from adjacent soil with values ranging from 0.01-0.6 mg.kg^{-1} (Table 1). Similarly, roots sampled from the river bank had significantly higher Ni values ranging from 0.6-2.9 mg.kg^{-1} when compared with 0.0-0.3 mg.kg^{-1} detected in roots sampled from adjacent soil (Table 1).

3.1.5 Cadmium (Cd)

Data collected from sites 2, 3 and 4 showed that Cd shoot concentrations of *P. australis* were significantly higher between those sampled from the river banks and adjacent soil. Cadmium in shoots from the river bank ranged from 0.01-56.6 mg.kg^{-1} compared with 0.01-2.3 mg.kg^{-1} recorded in shoot samples from adjacent soil (Table 1). In the roots, significant differences were in Cd samples at river banks in site 2, 3 and 4 as compared with those from adjacent soil (Table 1).

3.1.6 Lead (Pb)

Pb in shoots of *P. australis* analyzed from the river bank in all sites was significantly higher than their counterparts collected from the adjacent soil. From the river bank, Pb values ranged from 1.5-23.6 mg.kg^{-1} (Table 1). The highest concentration of Pb from the river bank was observed in site 3 (23.6 mg.kg^{-1}). Generally, low contents of Pb were found in tissues from adjacent soil (Table 1). The Pb content in roots was significantly different in three out of the 4 sites. The concentration of Pb in roots from the river bank ranged between 0.1-5.6 mg.kg^{-1} with site three recording the highest Pb concentration (5.6 mg.kg^{-1}). Most of roots sampled from the adjacent soil had the lowest Pb concentration ranging from 0.1-1.9 mg.kg^{-1} (Table 1).

3.1.7 Manganese (Mn)

The total content of Mn in shoots ranged from 1.6-97.3 mg.kg^{-1} and 0.8-35.9 mg.kg^{-1} in samples from river bank and the adjacent soil respectively. Highest Mn values were found in site 4, followed by sites 3, 2 and 1 respectively (Table 1). The root analysis result for Mn showed that three out of four sites had significant higher values of Mn in *P. australis* plant collected from river bank compared with those from adjacent soil. As observed in shoots, more Mn in roots was recorded in plant samples from the adjacent soil. Root values for Mn from river bank soil ranged from 0.8-33.7 mg.kg^{-1} (Table 1) and the values for plants from adjacent soil ranged from 0.4-10.3 mg.kg^{-1}. Compared with the values in shoots, highest Mn values in roots were found in sites 4, 3, 2 and 1 respectively.

3.1.8 Iron (Fe)

The mean value of Fe in shoot of *P. australis* samples collected from the 4 sites was significantly different from each other when data from the river bank and adjacent soil were compared. Levels of Fe from river bank samples ranged from 26.3-56.6 mg.kg^{-1}. The adjacent soil concentration of Fe in shoots ranged from 6.3-26.2 mg.kg^{-1}. The greatest Fe concentrations were also found in samples from the river bank compared with those from adjacent soils (Table 1).

The root accumulation of Fe in *P. australis* followed a similar pattern. Results indicated that Fe levels in most of the studied sites were greater in roots than shoots (Table 1). For instance, the highest concentration of 1164.4 mg.kg^{-1} Fe in the roots were recorded in site 4 but the shoot value in the same plant was 56.6 mg.kg^{-1}.

3.1.9 Copper (Cu)

The shoot and root concentrations of Cu in *P. australis* from the river bank were significantly higher than those from adjacent soil. Results showed that Cu values in shoots from the river bank ranged from 2.6-6.0 mg.kg^{-1}, but were significantly less in samples from adjacent soil, with values between 0.0 and 2.4 mg.kg^{-1} (Table 1).

Concentration of Cu in roots of *P. australis* were significantly different in three out of the four sites sampled (Table 1). Comparison across the sites showed that higher levels of Cu in roots were found at sites three and four (Table 1).

3.1.10 Zinc (Zn)

Zn in shoots of *P. australis* which were growing on the river banks were significantly higher compared with those growing in the adjacent soil in all sites. Concentration of Zn in shoots from the river bank and adjacent soil ranged from 3.8-50.6 mg.kg^{-1} and 0.0-10.1 mg.kg^{-1} respectively (Table 1). Shoots sampled from sites 4 and 3 accumulated more Zn than those sampled from site 2 and 1. Total Zn contents in roots of *P. australis* from the river bank and from the adjacent soil ranged from 6.2 -15.8 mg.kg^{-1} and 0.4-14.1 mg.kg^{-1} respectively (Table 1).

3.2 Chlorophyll Contents of P. australis Growing On the River Banks and in Adjacent Soil

Results of chlorophyll content showed that chlorophyll a, b and a+b were present in all the leaves examined from all the study sites (Figure 1). However, in the case of the river bank soil, the leaves from site three and one (7.9 and 8.8 mg.L^{-1}) recorded very low values for chlorophyll a+b (Figure 1). Whereas, the leaves from adjacent soil sites had greater values for chlorophyll a, b and a+b (Figure 1).

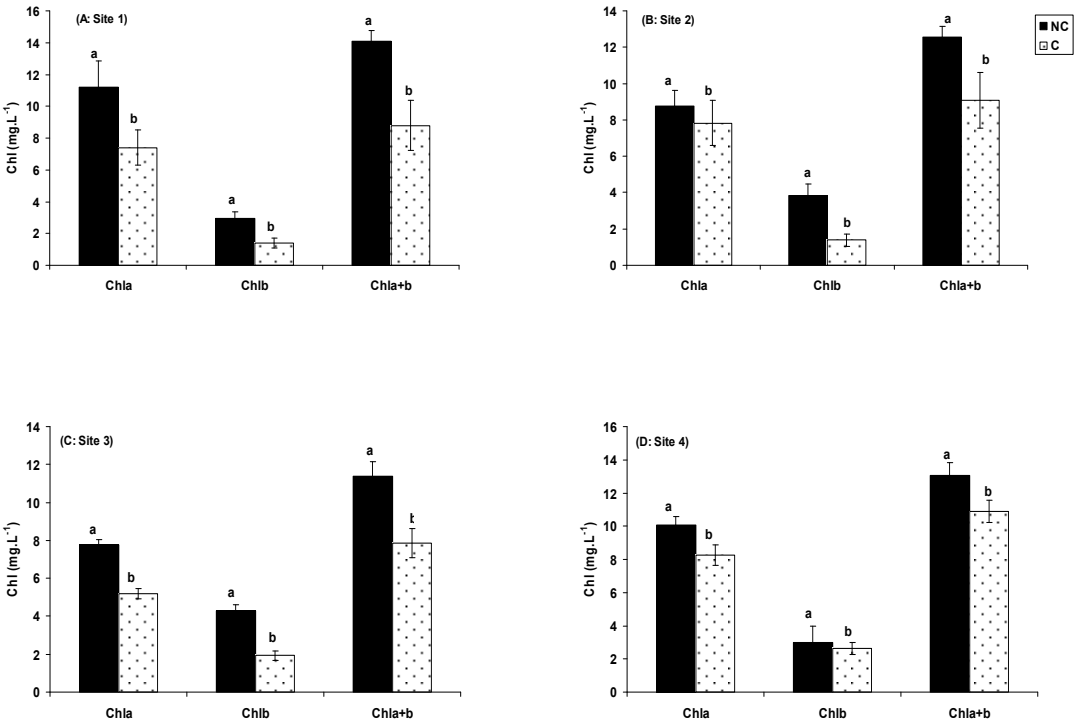

Figure 1. Effects of metal contaminations on Chl concentrations in plants sampled at A) Site 1; B) Sites 2; C) Site 3 and D) Site 4 along the lower Diep River, Milnerton, in the Western Cape Province. NB: NC-Adjacent soil, C-River bank

3.3 Measurement of Photosynthesis in P. australis Growing on the River Bank and in Adjacent Soil

Results showed that the photosynthesis values varied in plant leaves on both river bank and adjacent soil from site to site (Figure 2). Photosynthesis rate (A) ranged between 1.0-10.6 µmol, with plants from the river bank soil at site 1 exhibiting the highest rate of photosynthesis followed by plants from the other sites in the order 1 > 4 > 2 > 3. Similarly, in the adjacent soil, the values for photosynthesis (A) ranged from 6.5-13.4 µmol, in decreasing order of 1> 4> 2 > 3. Results also showed that rate of evapotranspiration (E) was significantly higher (2.1-2.3 µmol) for plants sampled in sites one and four, than at site two and three (1.3-1.7 µmol) (Figure 2). For both the river bank soil and in adjacent soil, the marginal difference in the values for evapotranspiration (E) is minimal; indicating that stomata function is insignificant.

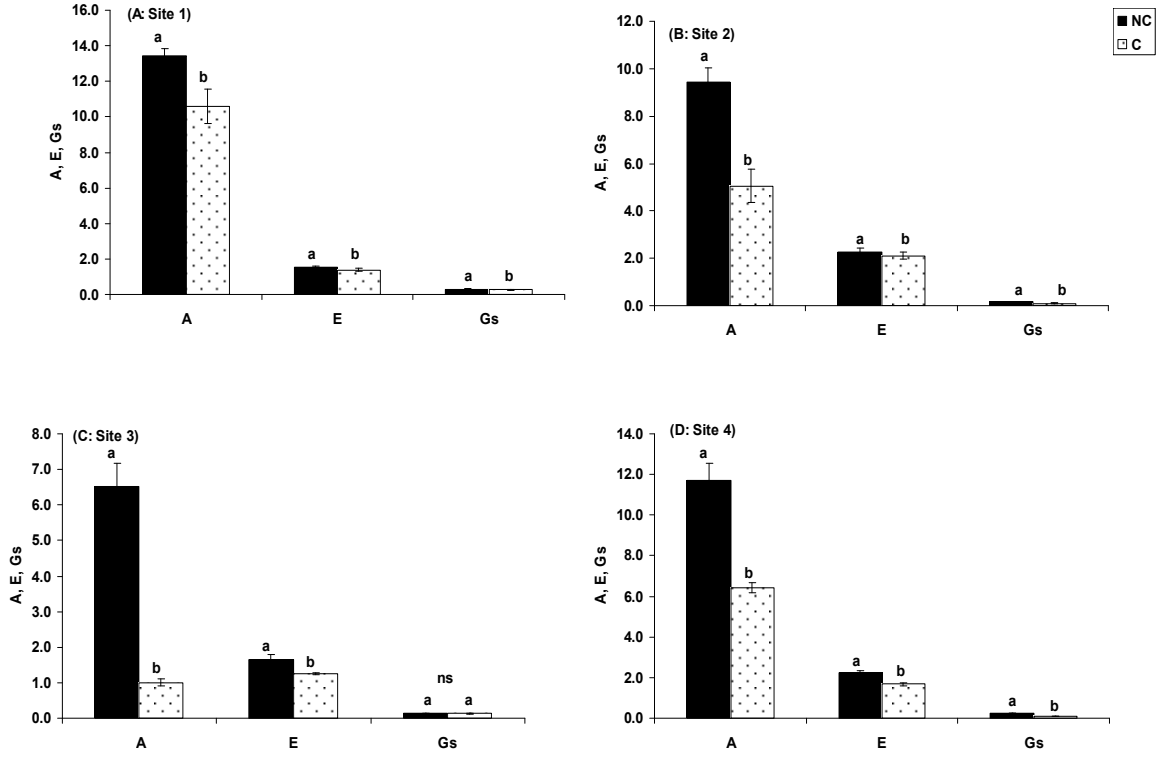

Figure 2. Effects of metals contamination on photosynthesis (A) (µ mol CO_2 $m^{-2}{\cdot}s^{-1}$), evapotranspiration (E) (mmol $H_2O{\cdot}m^{-2}{\cdot}s^{-1}$), and stomata conductance (Gs) (mmol$\cdot m^{-2}{\cdot}s^{-1}$) in plants sampled at A) Site 1; B) Sites 2; C) Site 3 and D) Site 4 along the lower Diep River, Milnerton in the Western Cape Province. NB: NC-Adjacent soil, C-River bank

4. Discussion

Results of this study revealed that plants from all the sites investigated were generally contaminated with all the metals measured. Al was the single highest metal measured in the shoots (Table 1) and roots of *P. australis* (Table 1), and this was followed by Pb, Ni and Cr in shoots. Cr, Co and Pb were also significantly higher in the root samples of plants from river bank than samples from the adjacent soil in some of the sites studied (Table 1).

The concentrations of Al in shoots of *P. australis* sampled from the river bank sites were in the range 23.7-237.9 $mg.kg^{-1}$. These were above the optimum recommended concentration level of 15-18 $mg.kg^{-1}$ (Dobermann & Fairhurst, 2000) for a closely related wetland plant species such as Rice. Shoot samples of *P. australis* from adjacent soils had Al concentrations ranging from 8.8-16.9 $mg.kg^{-1}$, which were below the recommended optimum of 18 $mg.kg^{-1}$, thus suggesting that *P. australis* growing in these soils may not be physiologically affected by Al toxicity. The Al concentration in *P. australis* roots collected from the river banks of three out of four sites were higher than the adjacent soils (Table 1).

Concentrations of Cr in shoots of *P. australis* sampled from the river bank, at all sites were in the range 0.1-1.2 $mg.kg^{-1}$, while in the root samples it ranged from 0.1-488.6 $mg.kg^{-1}$. The optimum recommended concentration for

plants is 0.03-0.29 mg.kg^{-1} (Burke et al., 2000), and for brown rice 0.16 mg.kg^{-1} (Lin, 1991) as well as lettuce 0.17 mg.kg^{-1} (Qishlaqi et al., 2007). Cr was completely absent in the roots of samples from both sites one and four and in the shoots from site four. All other concentrations were within the critical limit for both shoot and root in all the studied sites, except in the root of site three (488.6 mg·kg^{-1}). This value was the highest obtained, supporting literature that most wetland plants retain higher amounts of metals in their roots than in leaf tissue (Keller et al., 1998; Karpiscak et al., 2001; Stoltz & Greger, 2002; Shuping et al., 2011).

Cobalt was another important metal that was found to accumulate in excessive concentrations (16.9-55.9 mg.kg^{-1}) in *P. australis* shoots collected from the river bank and adjacent soil in site 4. Although small amounts were recorded at sites 1, 2 and 3, their levels (0.00-0.35 mg.kg^{-1}) were within the acceptable limits for plant tissues (Gough et al., 1979). At site 4 the observed tissue concentrations ranged from 16.9-55.9 mg.kg^{-1}, which were above the critical levels of 19-32 mg.kg^{-1} (Gough et al., 1979) in a closely related Sudan grass. The root levels across all sites ranged from 0.0-25.6 mg.kg^{-1}. However, highest levels (25.6 mg.kg^{-1}) were recorded only in plant roots collected from river bank four. This again confirms the findings of several wetland studies that the greatest metal accumulations occur in the roots (Karpiscak et al., 2001; Stoltz and Greger, 2002; Shuping et al., 2011).

Ni concentrations found in this study ranged from 0.01-1.9 mg.kg^{-1} in the shoots and 0.0-2.9 mg.kg^{-1} in the roots. The established threshold of Ni in different plant species range from 5-100 mg.kg^{-1} above which, plant physiological activities have been reportedly affected (Podlesakova et al., 2002). A comparison between the recorded Ni concentrations in this study and established threshold guidelines from the literature (Qishaqi et al., 2007) shows that they are below the established standards and may therefore not pose any toxic threat to *P. australis* in the studied ecosystem (Table 1).

In this study, Cd concentrations in both shoots and roots ranged 0.2-56.6 mg.kg^{-1} and 0.0-35.3 mg.kg^{-1}, respectively (Table 1), while the critical levels suggested by Kabata-Pendias and Pendias (1992) is 0.5-0.7 mg.kg^{-1}. Shoots sampled along the river bank showed that at site one (9.07 mg.kg^{-1}) and at site two (56.67 mg.kg^{-1}), Cd values were significantly higher at site three (0.27 mg.kg^{-1}) but lower than the critical level of 0.5-0.7 mg.kg^{-1} as proposed by Kabata-Pendias and Pendias (1992). Comparatively, shoots from the adjacent soil in sites 2, 3 and 4 had low Cd values which were below the critical limit except in site 1 (2.3 mg.kg^{-1}).

Roots sampled from the river bank in sites 1, 3 and 4 had low values of Cd below the critical levels whereas site two (35.3 mg.kg^{-1}) was above the recommended optimum. Compared to the adjacent soil, root values from site three and four were higher and above the established guidelines. Site one results 0.1 mg.kg^{-1} were far below the optimum recommended (Table 1).

In comparing the Cd concentrations reported in our study and the established threshold guidelines of Kabata-Pendias and Pendias (1993), results show that shoot from the river bank (75%) and adjacent soil (25%) sites had Cd values higher than the established standards by indicating that Cd was a significant pollutant in the Diep River ecosystem**.**

According to Burke et al. (2000), the critical concentration of Pb in plants range from 0.12-0.5 mg.kg^{-1}. The concentration of Pb in the shoots sampled from the river bank ranged from 1.5-23.6 mg.kg^{-1} (Table 1). All these values were above the established critical value. Shoots from the adjacent soils had Pb values ranging from 0.5-1.4 mg.kg^{-1}. Seventy five percent of the sites had elevated Pb values above the recommended optimum (Table 1). Taken together, results from this study suggest that the sites are heavily contaminated with Pb and may affect the growth of *P. australis* and other organisms found in the vicinity (Qishaqi et al., 2007).

Table 2 indicates that plants from the river bank soil of 3 out of 4 sites had elevated Pb levels ranging from 0.9-5.6 mg.kg^{-1}. From the adjacent soils, Pb levels in the roots ranged from 0.1-1.9 mg.kg^{-1} with 50% possessing values above the established limits by Canadian Council of Ministers of the Environment (CCME, 1999).

Total Mn in *P. australis* tissues ranged from 0.9-97.3 mg.kg^{-1} in shoots (Table 1) and 0.4-33.7 mg.kg^{-1} in roots (Table 1). The proposed threshold level for Mn in plants varies from 50-500 mg.kg^{-1} (Allen, 1989). In this study the highest Mn concentration (97.3 mg.kg^{-1}) was found in shoots sampled from the river bank in site 4. This is the only site which had excessive Mn that could lead to toxicity in *P. australis*.

Total Fe in the shoots and roots ranged from 6.3-56.6 mg kg^{-1} and 0.0-1164 mg.kg^{-1} respectively (Table 1). The established guideline for Fe content in different plant parts at which toxicity is expected is between 1100-1600 mg kg^{-1} for most wetland plants (Marschner, 1995). More Fe accumulated in roots than in shoots. Generally, plant tissues contained Fe below this recommended threshold value, indicating that Fe was probably not high enough to cause toxicity in *P. australis*.

Table 2. Concentrations ($mg.kg^{-1}$) of metals in plant roots sampled from four different soil sites in the Diep River

Site	Status of the sampled site	Al	Cr	Co	Ni	Cd	Pb	Mn	Fe	Cu	Zn
						$mg.kg^{-1}$					
1	Adjacent soil	1.0 ± 0.0^{a}	NIL	0.2 ± 0.0^{b}	0.0 ± 0.0^{b}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.4 ± 0.3^{a}	0.0 ± 0.0^{b}	1.6 ± 0.3^{b}	1.1 ± 0.7^{b}
	River bank	2.0 ± 0.0^{a}		0.5 ± 0.1^{a}	2.9 ± 1.3^{a}	0.0 ± 0.0^{a}	0.1 ± 0.0^{a}	0.8 ± 0.4^{a}	14.5 ± 7.7^{a}	2.9 ± 0.5^{a}	6.2 ± 2.4^{a}
	F- statistics	0.00^{NS}		6.90*	5.57*	0.01^{NS}	3.64^{NS}	0.74^{NS}	16.11***	2.66 **	4.27^{NS}
2	Adjacent soil	5.4 ± 2.3^{b}	0.0 ± 0.0^{b}	0.1 ± 0.0^{b}	0.3 ± 0.0^{a}	3.5 ± 1.1^{b}	0.5 ± 0.1^{b}	3.6 ± 0.9^{b}	22.9 ± 3.2^{b}	0.0 ± 0.0^{b}	0.4 ± 0.4^{b}
	River bank	26.9 ± 6.1^{a}	0.1 ± 0.1^{a}	0.2 ± 0.0^{a}	0.6 ± 0.2^{a}	35.3 ± 16.8^{a}	0.9 ± 0.1^{a}	5.3 ± 1.0^{a}	49.6 ± 11.4^{a}	2.6 ± 0.6^{a}	8.9 ± 2.5^{a}
	F- statistics	21.35***	6.2**	8.82***	2.2*	3.57*	4.64**	4.89**	5.11*	15.84***	11.38**
3	Adjacent soil	5.5 ± 1.7^{b}	12.5 ± 6.8^{b}	0.0 ± 0.0^{b}	0.1 ± 0.1^{b}	0.01 ± 0.0^{b}	1.9 ± 0.3^{a}	4.6 ± 0.9^{b}	1.2 ± 1.0^{b}	0.7 ± 0.2^{b}	14.1 ± 1.1^{b}
	River bank	25.6 ± 4.0^{a}	488.6±167.8a	0.7 ± 0.7^{a}	0.6 ± 0.3^{a}	0.12 ± 0.0^{a}	5.6 ± 1.3^{b}	6.9 ± 1.3^{a}	37.7 ± 11.6^{a}	3.1 ± 0.7^{a}	15.4 ± 2.4^{a}
	F- statistics	46.71***	8.04*	2.13**	9.5**	5.91*	7.91*	36.740*	9.71**	1.82**	99.84***
4	Adjacent soil	89.8 ± 19.7^{b}	NIL	5.5 ± 1.7^{b}	0.0 ± 0.0^{b}	0.01 ± 0.0^{b}	1.2 ± 0.1^{b}	10.3 ± 1.2^{b}	248.3 ± 46.8^{b}	0.3 ± 0.3^{b}	15.2 ± 1.7^{b}
	River bank	313.0 ± 46.1^{a}		25.6 ± 4.0^{a}	1.9 ± 0.5^{a}	0.30 ± 0.0^{a}	1.7 ± 0.8^{a}	33.7 ± 2.7^{a}	1164.4 ± 123.5^{a}	7.2 ± 1.3^{a}	15.8 ± 2.5^{a}
	F- statistics	19.81***		46.77***	4.64 **	0.71*	1.39 **	61.88***	48.16***	25.49***	76.86*

Mean ± SE, followed by dissimilar letter in the same column are significant at P=0.05 according to Fischer LSD. (*: $P \leq 0.05$, **: $P \leq 0.01$, ***: $P \leq 0.001$; NS = Not significant)

Total Cu in the shoots ranged from 0.0-6.0 mg kg^{-1} (Table 1) and corresponding values in roots ranged from 0.0-7.2 $mg.kg^{-1}$ (Table 1). The normal Cu concentration for plant growth in plant tissues is 5-20 $mg.kg^{-1}$ (Kabata-Pendias and Pendias, 1992). However, Marschner (1995) established that at 20-30 $mg.kg^{-1}$ Cu toxicity is likely to occur in shoots. All plant organs analysed from all sites in this study had lower concentrations of Cu.

Total Zn in shoots and roots of *P. australis* ranged from 0.0-50.6 $mg.kg^{-1}$ (Table 1) and 0.4-15.8 $mg.kg^{-1}$ (Table 1), respectively. Previous studies reported Zn phytotoxic levels for plants to vary from 100-1500 $mg.kg^{-1}$ (Chaney, 1989, Marschner, 1995). However, in the present study values below this range were obtained.

Significant differences were found in the values of Chl a, Chl b, and total chlorophyll (Chl a+b) in *P. australis* and higher Chl.τ values were obtained in *P. australis* plants growing in the adjacent soil compared with those collected from the river banks (Figure 1). Similarly, photosynthesis rate (A), evapotranspiration (E) intercellular carbon dioxide concentration (Ci) and stomatal conductance (Qs) in plants from the river bank were lower as compared to those measured in adjacent soil (Figure 1). The interference with the photosynthetic apparatus in plants leaves measured from the river bank was probably due to elevated levels of certain metals in the soil (Ayeni et al., 2010) (Table 1) which caused excessive metal accumulation in the tissues (Table 1), thus interfering with chlorophyll synthesis and photosynthesis. Table 3 also showed consistent with the values in the plant chlorophyll content.

Table 3. Comparison Chlorophyll concentration of studied plant (*Phragmites australis)* and other wetland plants

Species	Cholorophyll	References
Phragmites australis	11.82	Studied plant
Juncus roemerianus,	11.98	Stoddard et al., 2006
Spartina alterniflora	29.87	Stoddard et al., 2006
Rhizophora mangle	30.68	Stoddard et al., 2006

This is consistent with previous studies which reported that excessive metals (such as Zn, Cd, Ni, Al, Cu) in the plant tissue negatively affected chlorophyll synthesis and photosynthesis process (Godbold, 1984; Rai et al., 1991; Hussain et al., 1991; Vangronsveld & Clijsters 1992; Kahle 1993; Krupa et al., 1993a, b; Monni et al., 2001; Kalavrouziotis et al., 2007). The decrease in chlorophyll content was found in sunflower (Zengin & Munzuroglu, 2006) as well as in almond (Elloumi et al., 2007). The decline in chlorophyll content in plants growing adjacent to

the river bank compared with those at the river bank which were exposed to higher metal concentration was believed to be due to (a) inhibition of important enzymes, (b) impairment in the supply of Mg, Fe, Zn. A similar decease in chlorophyll content under heavy metal stress was discovered in cyanobacteria, unicellular chlorophytes (Chlorella), gymnosperms, such as *Picea abies* and angiosperms, such as *Zea mays*, *Quercus palustrus* and *Acer rubrum* (Siedlecka & Krupa, 1996).

5. Conclusion

The higher concentration values of the metals in shoots and roots of *P. australis* sampled from the river bank as compared to those taken from the adjacent soil obtained in this study may be attributable to contamination of the soil by industrial effluents emanating from the industries in the area (Ayeni et al., 2010). This was reflected in the reduced photosynthesis and chlorophyll synthesis in plants which were growing close to the river bank. These physiological responses to metal contamination may thus possibly be used as biomarkers, in a biomonitoring programme.

Acknowledgements

We thank the National Research Foundation and the Cape Peninsula University of Technology for financial assistance. We also thank Mrs Rossouw of Stellenbosch University for assistance with the metal analysis.

References

Abdurakmanova, Z., Dzhumaev, B., & Abdullaev, A. (2000). Photosynthetic carbon metabolism in pea leaves under High Mountain visible and UV radiation. *Russian Journal of Plant Physiol*ogy*, 47*, 513-517.

Allen, S. E. (1989). *Analysis of ecological materials* (2nd ed.). Oxford: Blackwell Scientific Publications.

Arnon, D. I. (1949). Copper enzymes in isolated chloroplasts, Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology, 24*, 1-15. http://dx.doi.org/10.1104/pp.24.1.1

Ayeni, O. O., Ndakidemi, P. A., Snyman, R. G., & Odendaal, J. P. (2010a). Chemical biological and physiological indicators of metal pollution in wetlands. *Scientific Research and. Essays, 5*(15), 1938-1949.

Ayeni, O. O., Ndakidemi, P. A., Snyman, R. G., & Odendaal, J. P. (2010b). Metal contamination of soils collected from four different sites along the lower Diep River, Cape Town, South Africa. *International Journal of Physical Sciences, 5*(12), 2045-2051.

Baldantoni, D., Ligrone, R., & Alfani, A. (2009). Macro- and trace elements concentrations in leaves and roots of *Phragmites australis* in a volcanic lake in Southern Italy. *Journal of Geochemical Exploration, 101*, 166-174. http://dx.doi.org/10.1016/j.gexplo.2008.06.007

Bonanno, G., & Lo Giudice, R. (2010). Heavy metal bioaccumulation by the organs of *Phragmites australis* (common reed) and their potential use as contamination indicators. *Ecological Indicators, 10*, 639-645. http://dx.doi.org/10.1016/j.ecolind.2009.11.002

Bragato, C., Schiavon, M., Polese, R., Ertani, A., Pittarelo, M., & Malagoli, M. (2009). Seasonal variations of Cu, Zn, Ni, and Cr concentration in *Phragmites australis* in a constructed wetland of North Italy. *Desalination, 246*, 35-44. http://dx.doi.org/10.1016/j.desal.2008.02.036

Brown, C., & Magoba, R. (2009). *Rivers and wetlands of Cape Town: Caring for our rich aquatic heritage, Gezina*, South Africa: Water Research Commission. (pps. 1-68; 159-179).

Burke, D. J., Weis, J. S., & Weis, P. (2002). Release of metals by the leaves of the salt Marsh grasses *Spartina alterniflora* and *Phragmites australis*. *Estuaries Coast Shelf Science, 51*, 153-159. http://dx.doi.org/10.1006/ecss.2000.0673

Calheiros, C. S. C., Rangel, A. O. S. S., & Castro, P. M. L. (2007). Constructed wetland systems vegetated with different plants applied to the treatment of tannery waste water. *Water Research, 41*(8), 1790-1798. http://dx.doi.org/10.1016/j.watres.2007.01.012

Chaney, R. L. (1989). Toxic element accumulation in soils and crops: Protecting soil fertility and agricultural food-chains. In Bar-Yosef B, Barrow NJ, Goldshmid J (Eds*.). Inorganic Contaminants in the Vadose Zone.* (pp. 140-158). Springer-Verlag, Berlin. http://dx.doi.org/10.1007/978-3-642-74451-8_10

Clijsters, H., & Van Assche, F. (1985). Inhibition of Photosynthesis by heavy metals. *Photosynthesis Research, 7*, 31-40. http://dx.doi.org/10.1007/BF00032920

Cronk, K. J., & Fennessy, M. S. (2001). Wetland Plants: Biology and Ecology. Boca Raton, Fl.: Lewis Publishers. http://dx.doi.org/10.1201/9781420032925

Dahmani-Muller, H., Van Oort, F., Gelie, B., & Balabane, M. (2000). Strategies of heavy metal uptake by three plant species growing near a metal smelter. *Environmental Pollution, 109*, 231-238. http://dx.doi.org/10.1016/S0269-7491(99)00262-6

Dobermann, A., & Fairhurst, T. (2000). Rice nutrient disorders & nutrient management. Handbook series. Potash & Phosphate Institute (PPI), Potash & Phosphate Institute of Canada (PPIC) and International Rice Research Institute (IRRI). pp.191.

Dong, Q., Lawrence, C. J., Schlueter, S. D., Wilkerson, S. K., Lushbough, C., & Brendel, V. (2005). Comparative plant genomics at plant GDB; American Society of Plant Biology. *Plant Physiology, 139*, 610-618. http://dx.doi.org/10.1104/pp.104.059212

Elloumi, N., Ben, F., Rhouma, A., Ben, B., Mezghani, I., & Boukhris, M. (2007). Cadmium induced growth inhibition and alteration of biochemical parameters in almond seedlings grown in solution culture. *Acta Physiology of Plant, 29*, 57-62. http://dx.doi.org/10.1007/s11738-006-0009-y

Godbold, D. L., Horst, W. J., Collins, J. C., Thurman, D. A., & Marschner, H. (1984). Accumulation of zinc and organic acids in roots of zinc tolerant and non-tolerant ecotypes of *Deschampsia caespitose*. *Journal of Plant Physiology, 116*, 59-69. http://dx.doi.org/10.1016/S0176-1617(84)80084-X

Gough, L. P, Shacklette, H. T., & Case, A. A. (1979). *Element concentrations toxic to plants, animals and man.* US Department of Inter geological survey, Geological survey bulletin, 1466, Washington, DC.

Hiscox, J. D., & Israelstam, G. F. (1979). A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany, 57*, 1332-1334. http://dx.doi.org/10.1139/b79-163

Hussain, I., Raschid, L., Hanjra, M. A., Marikar, F., & Van der Hoek, W. (2001). *A framework for analyzing socioeconomic, health and environmental impacts of wastewater use in agriculture in developing countries*. Working Paper 26. Colombo: International Water Management Institute (IWMI).

Hussein, Y., Singh, A. K., Rai, L. C. (1991). Cadmium toxicity to photosynthesis and associated electron transport system of *Nostoc linckia*. *Buletin of Environment Contamination & Toxicology, 46*, 146-150. http://dx.doi.org/10.1007/BF01688268

Kabata-Pendias, A., & Pendias, H. (1992). *Trace elements in soils and plants* (2nd ed.). Boca Raton, Florida: CRC Press. pp. 365.

Kabata-Pendias, A., & Pendias, H. (2001). *Trace element in soil and plants* (3rd ed.). Boca Raton, Florida: CRC Press. pp. 413.

Kadlec, R. H., Knight, R. L., Vymazal, L., Brix, H., Cooper, P., & Haberi, R. (2000). Constructed wetlands for pollution control processes. Performance, design and operation. IWA Scientific and Technical report No. 8, IWA Publishing London, UK.

Kahle, H. (1993). Responses of roots of trees to heavy metals. *Environmental Experimental Botany, 33*(1), 99-119. http://dx.doi.org/10.1016/0098-8472(93)90059-O

Kalavrouziotis, I. K., Koukoulakis, P. H., Robolas, P., Papadopoulos, A. H., & Pantazis, V. (2007). Interrelationships of heavy metals macro and micronutrients, and properties of a soil cultivated with *Brassica oleracea var. italica* (Broccoli), under the effect of treated municipal wastewater. *Water, Air, and soil Pollution, 190*, 309-321. http://dx.doi.org/10.1007/s11270-007-9602-y

Karpiscak, M. M., Whiteaker, L. R, Artiola, J. F., & Foster, K. E. (2001). Nutrient and heavy metal uptake and storage in constructed wetland systems in Arizona. *Water Science Technology, 44*, 455-462.

Keller, B., Lajtha, K., & Cristofor, S. (1998). Trace metal concentrations in the sediments and plants of the Danube delta, Romania. *Wetland, 18*, 42-50. http://dx.doi.org/10.1007/BF03161441

Khalid, B. Y., & Tinsley, J. (1980). Some effects of nickel toxicity on rye grass. *Plant and Soil, 55*(1), 139-144. http://dx.doi.org/10.1007/BF02149717

Krupa, Z., Quist, G., & Huner, N. P. A. (1993b). The effects of cadmium on photosynthesis of *Phaseolus vulgaris* - a fluorescence analysis. *Physiology Plantaterium, 88*, 626-630. http://dx.doi.org/10.1111/j.1399-3054.1993.tb01381.x

Krupa, Z., Siedlecka, A., Maksymiec, W., & Baszynski, T. (1993a). In vivo response of photosynthetic apparatus of *Phaseolus vulgaris* L. to nickel toxicity. *Journal of Plant Physiology, 142,* 664-668. http://dx.doi.org/10.1016/S0176-1617(11)80899-0

Kucuk, O. S., Sengul, F., & kapdan, I. K. (2003). Removal of ammonia from tannery effluents in a reed bed constructed wetland. *Water Science and Technology, 48*(11-12), 179-186.

Laing, G. D., Tack, F. M. G., & Verloo, M. G. (2003). Performance of selected destruction methods for the determination of heavy metals in reed plant (*Phragmites australis*). *Analytical. Chim. Acta, 479*, 191-198. http://dx.doi.org/10.1016/j.aca.2003.08.044

Lin, H. T. (1991). A study on the establishment of heavy metal tolerance in soil through the heavy metal concentration of crop. Unpub. M.Sc. Thesis. Research Institute of soil science, National Chung Hsing University, Taichung, Taiwan.

Liphadzi, M. S., & Kirkham, M. B. (2006). Heavy-metal displacement in chelate -treated soil with sludge during phytoremediation. *Journal of Plant Nutrition and Soil Scence, 169*(6), 737-744. http://dx.doi.org/10.1002/jpln.200622005

Liphadzi, M. S., & Kirkham, M. B. (2005). Phytoremediation of soil contaminated with heavy metals: a technology for rehabilitation of the environment. *South African Journal of Botany, 71*, 24-37. http://dx.doi.org/10.1016/S0269-7491(00)00125-1

Marschner, H. (1995). *Mineral nutrition of higher plants* (2nd ed.). London: Academic Press. pp 889.

Monni, S., Uhlig, C., Hansen, E., & Magel, E. (2001). Ecophysiological responses of *Empetrum nigrum* to heavy metal pollution. *Envronmental Pollution, 112*, 121-129.

Nagel, K., Adelmeier, U., & Voigt, J. (1996). Sub cellular distribution of cadmium in the unicellular green alga *Chlamydomonas reinhardtii. Journal of Plant Physiology, 149*, 86-90. http://dx.doi.org/10.1016/S0176-1617(96)80178-7

Odendaal, J. P., & Reinecke, A. J. (1999). The sublethal effects and accumulation of cadmium in the terrestrial isopod *Porcellio laevis* Latr (Crustacea isopoda). Archives of *Environmental Contamination and. Toxicology, 36*, 64-69. http://dx.doi.org/10.1007/s002449900443

Pflugmacher, S., Weigand, C., Beattle, K. A., Krause, E., Steinberg, C. E. W, & Codd, G. A. (2000). Uptake, effects and metabolism of cyanobacterial toxins in the emergent reed plant *Phragmites australis* (Cav.) Trin Ex-steud. *Environmental Toxicology and Chemistry, 20*(4), 846-852.

Plekhanov, S. E,, Chemeris, Y. K. (2003). Early toxic effects of zinc, cobalt, and cadmium on photosynthetic activity of the Green alga *Chlorella pyrenoidosa* Chick S-39. *Biology Bulletin, 30*(5), 506-511. http://dx.doi.org/10.1023/A:1025806921291

Podlesakova, J., Memecek, R., & Vacha, R. (2002). Critical values of trace elements in soils from the viewpoint of the transfer pathway soil-plant. *Research institute for soil and water conservation, Prague, Czech Republic, 48*(5), 193-202.

Prasad, M. N. V., & Strzalka, K. (2000). Impact of heavy metals on photosynthesis. In Heavy Metal stress in plants; from molecules to ecosystems, (Eds.), Prasad MNV and Hagemeyer J, Springer, Berlin. pp 117-138.

Qishlaqi, A., Moore, F., & Forghani, G. (2007). Impact of untreated wastewater irrigation on soils and crops in Shiraz suburban area, Sw, Iran. *Environmental Monitoring and Assessment, 141*, 257-273. http://dx.doi.org/10.1007/s10661-007-9893-x

Rai, L. C., Singh, A. K, & Mallick, N. (1991). Studies on photosynthesis, the associated electron transport system and some physiological variables of *Chlorella vulgaris* under heavy metal stress. *Journal of Plant Physiology, 137*, 419-424. http://dx.doi.org/10.1016/S0176-1617(11)80310-X

Rodríguez, M. C., Barsanti, Passarelli, Evangelista, V., Conforti, V., & Gualtieri, P. (2007). Effects of chromium on photosynthesis and photoreceptive apparatus of the alga *Clamydomonas reinhardtii*. *Environment Research, 105*, 234-239.

Saltonstall, K. (2008). Common Reed: *Phragmites australis*: Fact Sheet Plant conservation alliance alien plant working group. Retrieved from http://www.nps.gov/plants/ALIEN/facts/phaul.htm (October 7, 2008).

Schoefs, B. (2001). The protochlorophyllide-chlorophyllide cycle. *Photosynthesis Research, 70*(3), 257-271. http://dx.doi.org/10.1023/A:1014769707404

Sheoran, I. S., Singal, H. R., & Singh, R. (1989). Effect of Cadmium and Nickel on photosynthesis and enzymes of the photosynthetic carbon reduction cycle in pigeon pea (*Cajanus cajan.* L.) *Photosynthesis Research, 23*, 345-351. http://dx.doi.org/10.1007/BF00034865

Siedlecka, A., & Krupa, Z. (1996). Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaselus vulgaris*. *Plant Physiology and Biochemistry, 34*, 833-841.

Stobart, A. R., Griffiths, W. T., Ameen-Bukhari, J., & Shewood, R. P. (1985). The effect of Cd on the biosynthesis of chlorophyll in leaves of barley. *Physiology of Plant, 63*, 293-298. http://dx.doi.org/10.1111/j.1399-3054.1985.tb04268.x

Stoddard, J. L., Larsen, D. P., Hawkins, C. P., Johnson, R. K., & Norris, R. H. (2006). Setting expectations for the ecological condition of streams: the concept of reference condition. *Ecological Applications, 16*, 1267-1276. http://dx.doi.org/10.1890/1051-0761(2006)016[1267:SEFTEC]2.0.CO;2

Stoltz, E., & Gregor, M. (2002). Accumulation properties of As, Cd, Cu, Pb and Zn by four wetland plant species growing in submerged mine tailings. *Environmental and Experimental Botany, 47*, 271-280. http://dx.doi.org/10.1016/S0098-8472(02)00002-3

Sun, E., & Wu, F. (1998). Along-vein necrosis as indicator symptom on water spinach caused by nickel in water culture. *Botanical Bulletin of Academia Sinica, 39*, 255-259.

Vangronsveld, J., & Clijsters, H. (1992). A biological test system for the evaluation of metal phytotoxicity and immobilization by additives in metal - contaminated soils. In: Merian, E and Haerdi W (Eds), *Metal Compounds in Environment and Life, 4 (Interrelation Between Chemistry and Biology)*. *Science and Technology Letters,* Northwood, pp. 117-125.

Vassilev, A. D., Kapulnik, Y., Raskin, I., & Salt, D. E. (1998). The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiology, 117*, 447-453. http://dx.doi.org/10.1104/pp.117.2.447

Vázquez, S., Goldsbrough, P., & Carpena, R. O. (2006). Assessing the relative contributions of phytochelatins and the cell wall to cadmium resistance in white lupin. *Physiology of Plant, 128*, 487-495.

Walker, D. J., Clemente, R., Roig, A., & Bernal, M. P. (2003). The effects of soil amendments on heavy metal bioavailability in two contaminated Mediterranean soils. *Environmental Pollution, 122*, 303-312. http://dx.doi.org/10.1016/S0269-7491(02)00287-7

Wetzel, R. G. (1995). Death, detritus and energy flow in aquatic ecosystems. *Freshwater Biology, 33*, 83-88. http://dx.doi.org/10.1111/j.1365-2427.1995.tb00388.x

Zayed, A., Little, C. M, Jin-Hong, Q., & Qian, J. H. (1998). Chromium accumulation, translocation and chemical speciation in vegetable crops. *Planta, 206*, 293-299. http://dx.doi.org/10.1007/s004250050403

Zengin, F. K., & Munzuroglu, O. (2006). Toxic effects of cadmium on metabolism of sunflower (*Helianthus annus* L) seedlings. *Acta Agricultural Scandinavia Sect. B, Plant and Soil Science, 56*, 224-229.

Zhang, X., Liu, P., Yang, Y. S., & Xu, G. (2007). Effect of Al in soil on photosynthesis and related morphological and physiological characteristics of two soybean genotypes. *Boston Studies in the Philosophy of Science, 48*, 435-44.